PATHOLOGY OF SEA LAMPREY INFLICTED WOUNDS ON RAINBOW TROUT

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PATHOLOGY OF SEA LAMPREY INFLICTED WOUNDS ON RAINBOW TROUT

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ABSTRACT

Rainbow trout (*Salmo gairdneri*) held in the laboratory were subjected to sea lamprey (*Petromyzon marinus*) attack for prescribed time periods to provide wound samples at different developmental stages up to 10 days. A lamprey destroys the epidermis in as little as 4 h, penetrates the dermis from 12 h to 2 days, and damages a large area of muscle from 2 to 10 days.

The wound healing process was examined by collecting tissue samples of the healing wound at prescribed times up to 3 months. Epithelialization of the wound area was complete by 2 weeks at which time heavy fibrogranulation tissue encompassed the area of dermal penetration and damaged muscle. The wound area had not returned to normal by 3 months as was evidenced by the fibroblastic activity which remained.

INTRODUCTION

The decline of major fish populations in the Great Lakes has been attributed in part to parasitism by the sea lamprey (*Petromyzon marinus*). Although chemical control has successfully reduced sea lamprey populations, the incidence of scarred fish taken in field surveys and by sport and commercial fishermen indicate significant numbers of lamprey remain in the Great Lakes.

Relatively little is known of the pathology of sea lamprey wounds in fish. Although sea lamprey attacks are frequently lethal (Lennon 1954), some fish survive multiple wounds as evidenced by scars found on large trout and salmon.

Information is needed on the pathology of sea lamprey wounds in fish and the wound healing process. In addition to improving knowledge of the host-parasite relationship, the data gathered should aid in the determination of the age of wounds and scars found in field surveys, thus providing a means to estimate the intensity of sea lamprey predation on Great Lakes fish stocks. Although King and Edsall (1979) described the gross pathology of sea lamprey marks on lake trout, information on the histopathology is lacking.

Rainbow trout (*Salmo gairdneri*) held in the laboratory were subjected to lamprey attacks for prescribed time periods to provide wounds at various stages of development. Observations were made on the areas of the integument and underlying muscle which were affected as the wound progressed, and on the extent of damage that occurred. Histopathological sections were also prepared from trout that had healed for prescribed time periods to provide information on the wound healing process.

METHODS AND MATERIALS

Experimental Animals

The rainbow trout used in the study were obtained from the Midwest Trout Farm, Harrison, Michigan. Before experimentation the fish were held in 1,000 l circular tanks which received continuously flowing 12°C well water of pH 7.1, hardness 330 mg $CaCO_3$/l and alkalinity 325 mg $CaCO_3$/l. Dissolved oxygen concentration was 9.0 ppm or higher. The fish were fed Trout Chow (Ralston Purina, Checkerboard Square, St. Louis, MO) *ad libitum*. The averages for length and weight of trout used in the wound development study were 30.6 cm and 309.1 g; the averages for those in the wound healing group were 31.1 cm and 328.4 g.

The sea lampreys obtained from the U.S. Fish and Wildlife Service laboratory at Hammond Bay, Michigan, were young adults that had recently completed metamorphosis. The lampreys were maintained in 150 l tanks receiving the same flowing well water as that used for the trout and were allowed to feed on carp (*Cyprinus carpio*) before experimentation. Lampreys used in the wound development study were 19.1 cm and those in the wound healing study were 22.9 cm in mean length.

Experimental Procedure

Individual trout were weighed, measured (total length), and placed singly in a 150 l fiberglass tank containing several sea lampreys. When one of the lamprey attached to the trout, the time and position of attachment were noted and the remaining lampreys were removed to a holding tank. The lamprey was allowed to attach to the trout for a prescribed time after which the lamprey and trout were anesthetized in 80 ppm tricaine methane sulfonate (MS-222), separated, weighed, and measured. The wound was then measured and photographed. The sequential development of lamprey wounds was followed by collecting tissue samples from wounded fish at 4 h, 12 h, 2 days, and 10 days after the initial lamprey attachment. Five fish were used for each sampling period. The wounds were classified by the method of King and Edsall (1979).

The trout used for wound healing were detached from the lampreys by anesthesia in MS-222 after 8 days and returned to holding tanks for the prescribed time period. Tissue samples from wounded fish were collected at 2 days, 2 weeks, 1 month, and 3 months after lamprey detachment. Five fish were used in each sample group.

A tissue sample was taken by cutting a rectangular section across the center of the wound. The samples were fixed immediately in 10% buffered neutral formalin and were then embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H & E). For some wound sections that exhibited fibrogranulation, a second slide was prepared and stained with Mallory's trichrome to demonstrate fibrosis (Luna 1960). The oral disk wound diameters averaged 2.10 × 1.83 cm in the wound development study and 2.62 × 2.15 cm in the wound healing study.

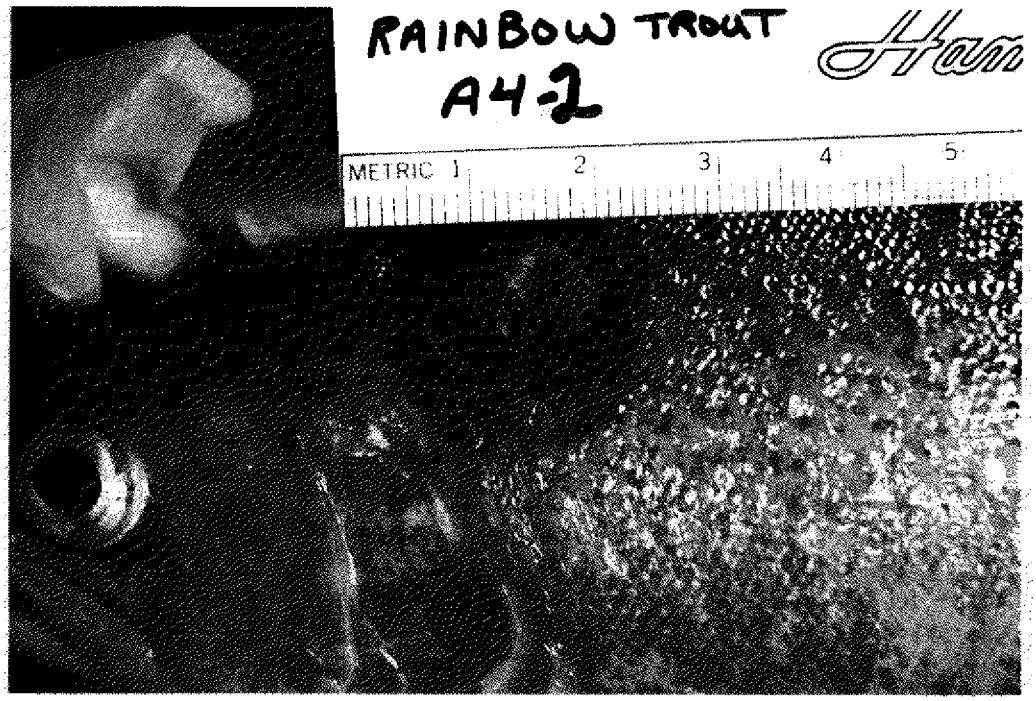

FIG. 1. Sea lamprey wound on a rainbow trout 4 h after attachment.

RESULTS AND DISCUSSION

WOUND DEVELOPMENT

GROSS PATHOLOGY

The central zone of the lamprey wound was generally elevated where the tongue had penetrated the skin.

Four Hour Wound (Type B, Stage I): Wounds were elevated and had one to three white abraded areas averaging 2.0 mm in diameter where the lamprey's tongue had penetrated the skin into the superficial levels of the dermis. The peripheral part of the wound, which was covered by the oral disk of the lamprey, lacked scales and showed slight to dark discoloration sometimes brown in color (Fig. 1).

Twelve Hour Wound (Type B, Stage I): The central portion was not significantly enlarged (average 2.0 mm) but the depth of the wounds had increased with penetration to the lower dermis and in one wound into the muscle (Type A, Stage I). Petechial hemorrhaging was evident on one fish and a more severe hemorrhaging occurred where the tongue had penetrated to the muscle. The peripheral portion of the wound lacked scales and showed discoloration; also at times a dark brown color was evident (Fig. 2).

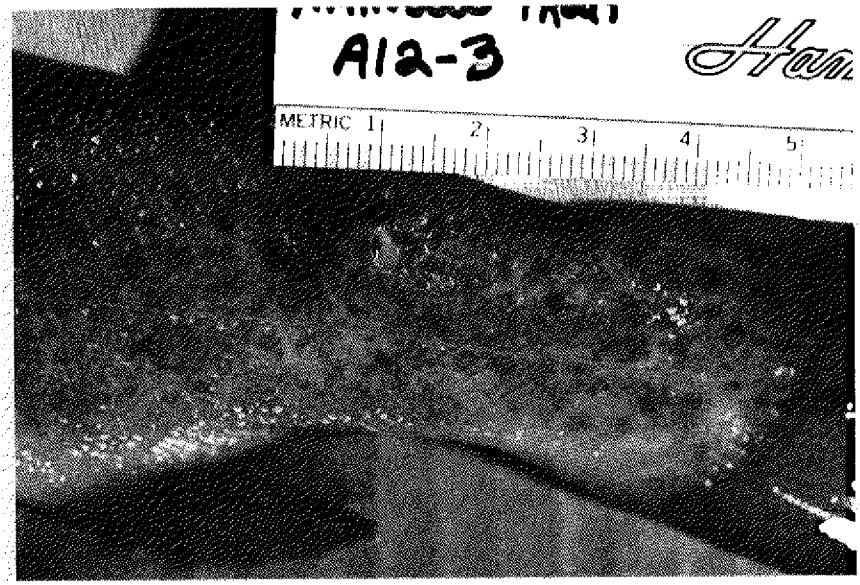

FIG. 2. Sea lamprey wound on a rainbow trout 12 h after attachment.

Two Day Wound (Type A, Stage I): The central portion of the wounds had increased slightly from 2.0 to 3.0 mm where the lamprey's tongue had penetrated through the dermis to the muscle. The peripheral portion of the wound area lacked scales and exhibited dark discoloration with some white interlaced areas of abrasion. The same brown coloration of the area was evident at times (Fig. 3).

Ten Day Wound (Type A, Stage I): The central portion of the wounds had one to three areas of dermal penetration to the muscle which ranged from 2.0 to 6.0 mm across with some ulceration occurring in the necrotic muscle in the larger dermal openings. The peripheral portion of the wound was lacking scales and was bleached to a white abraded area which frequently showed petechial hemorrhaging. At times a few small penetrations through the dermis to the muscle were evident (Fig. 4).

Lennon (1954) found that a sea lamprey attaches to a fish by strong suction which brings the skin of its host into contact with the lamprey's toothed tongue. A rocking motion of the toothed tongue then rasps a feeding hole through the skin and tissues of the host. This motion continues until a flow of blood is obtained. He also observed that many new wounds showed the application and pattern of buccal dentition. The suction exerted brings the disk teeth into close application with the surface of the fish, and there the cusps penetrate the skin or scales to help anchor the lamprey.

Lennon (1954) measured the diameter and depth of wounds in lake and

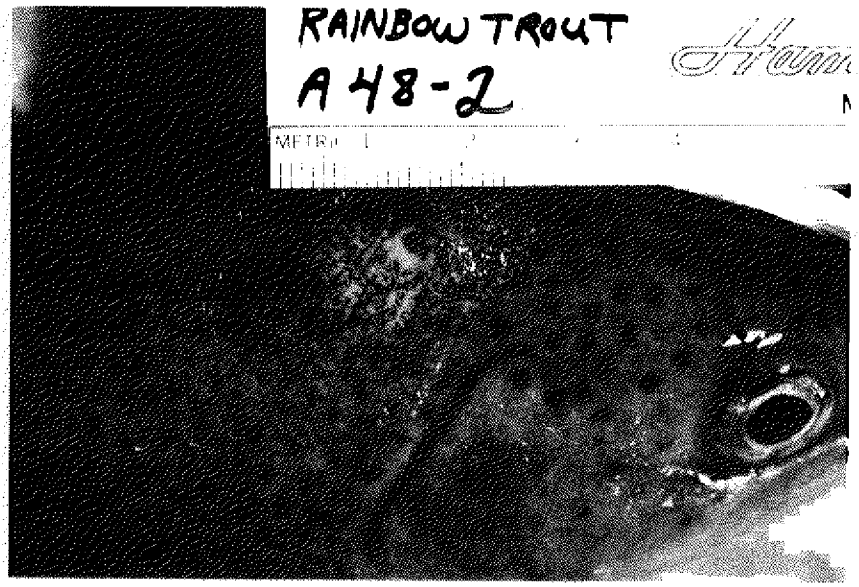

FIG. 3. Sea lamprey wound on a rainbow trout 2 days after attachment.

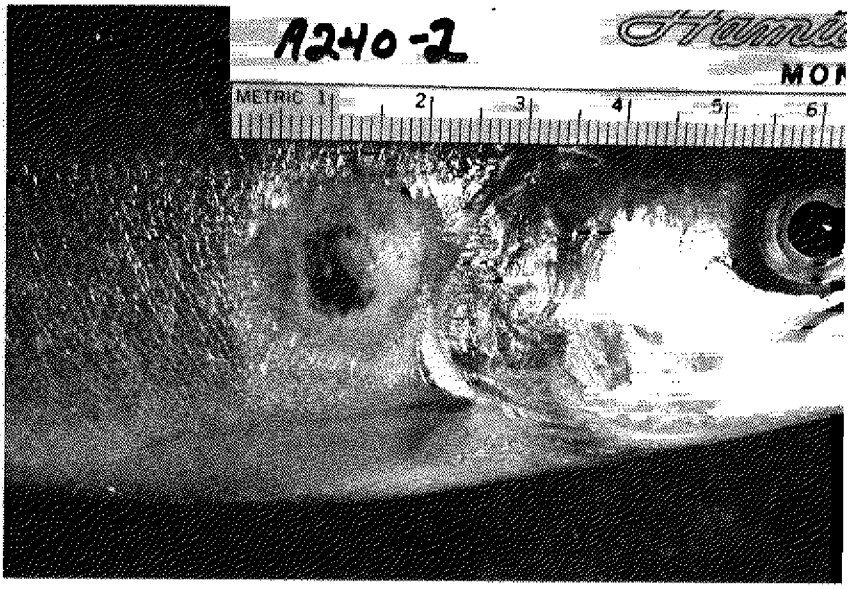

FIG. 4. Sea lamprey wound on a rainbow trout 10 days after attachment.

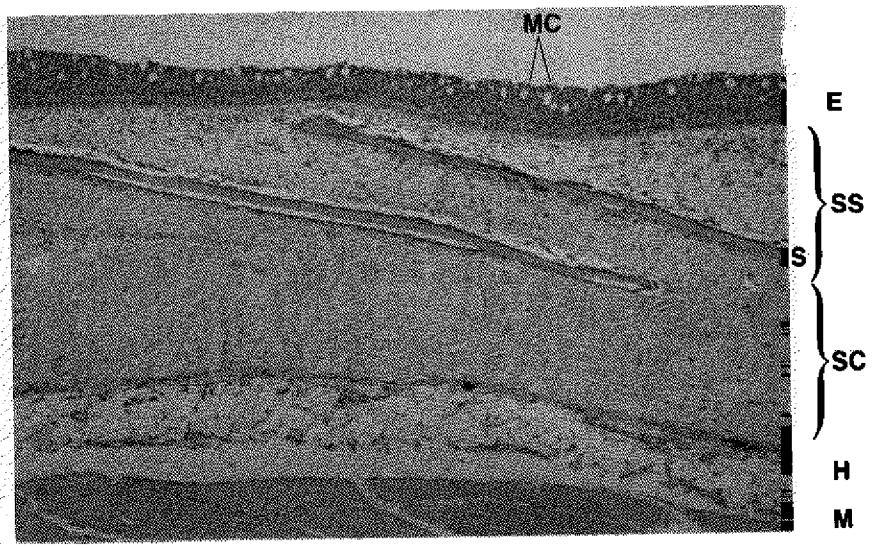

FIG. 5. Normal integument from a rainbow trout. Mucous cells (MC), Epidermis (E), Stratum spongiosum (SS), Scales (S), Stratum Compactum (SC), Hypodermis (H), Muscle (M), H & E ×40.

laboratory fishes and found no definite relation between size of the lamprey and size of wounds. He found that the area and depth of the wound depended on the attack duration, the amount of rasping by the tongue, the extent of tissue lysis caused by the action of the buccal gland secretion, the attack location on the fish, and the amount of movement by the lamprey from the original site of attachment.

HISTOPATHOLOGY

A section of normal fish integument is shown in Fig. 5 for comparison with the changes that occurred as a result of sea lamprey attack. The normal integument shows the epidermis with its complement of mucous cells. The dermis which lies below the epidermis consists of two zones. The stratum spongiosum is the germinal area where the scales originate. The stratum compactum contains dense layers of collagen fibers. The hypodermis contains a heavy concentration of lipid.

Four Hour Wound (Type B, Stage I): Most of the wound area lacked scales, the epithelium was essentially absent, or where present varied from ragged remnants to nests of necrotic basophilic material sometimes interspersed with mucous cells (Fig. 6).

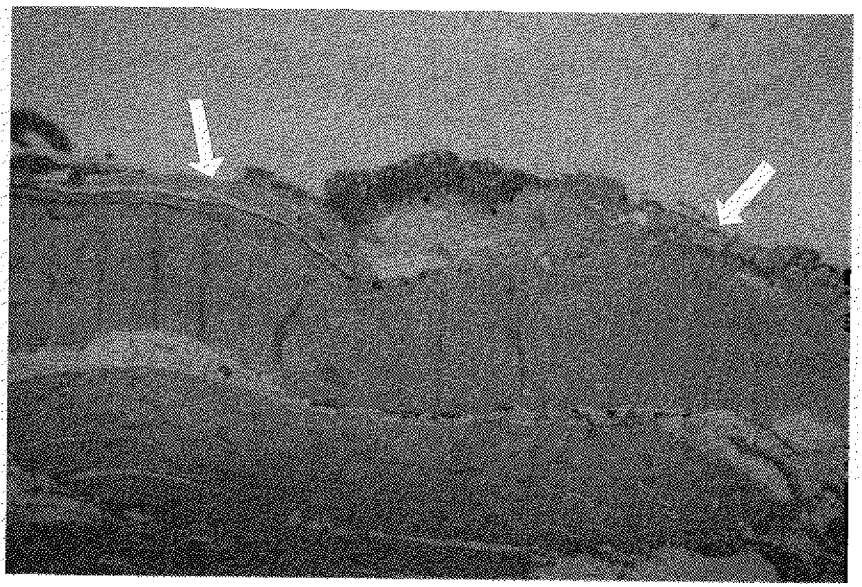

FIG. 6. Wound area after 4 h of sea lamprey attachment. Epithelial elements are essentially absent. H & E. ×25.

Much of the dermal stratum spongiosum had undergone caseation necrosis, and was somewhat basophilic. The dermal stratum compactum, hypodermis, and muscle all appeared normal.

Twelve Hour Wound (Type B, Stage I): In addition to the epidermal lesions noted at 4 h, the remaining epidermis at times exhibited caseation necrosis.

Besides the changes noted in the dermal stratum spongiosum described for the 4 h wound, some areas of the stratum spongiosum were vacuolated. Some areas of the wound lacked scales and in others scales were uplifted (Fig. 7). In one wound, rod shaped bacteria were located in the stratum spongiosum; in another wound evidence of hemorrhage was noted on the surface of the wound area. One wound penetrated the entire dermis and included a blood clot. This was the first appearance of a Type A, Stage I wound.

Damage in the dermal stratum compactum was generally confined to the superficial area with the exception of one Type A, Stage I wound which penetrated the dermis causing caseation necrosis (Fig. 8). The damage that occurred in the upper stratum compactum varied from a slight architectural disturbance to a limited caseation necrosis. The hypodermis appeared normal except in one wound in which it was disrupted where the Type A, Stage I wound penetration occurred.

The longitudinal muscle below and the damaged stratum compactum varied

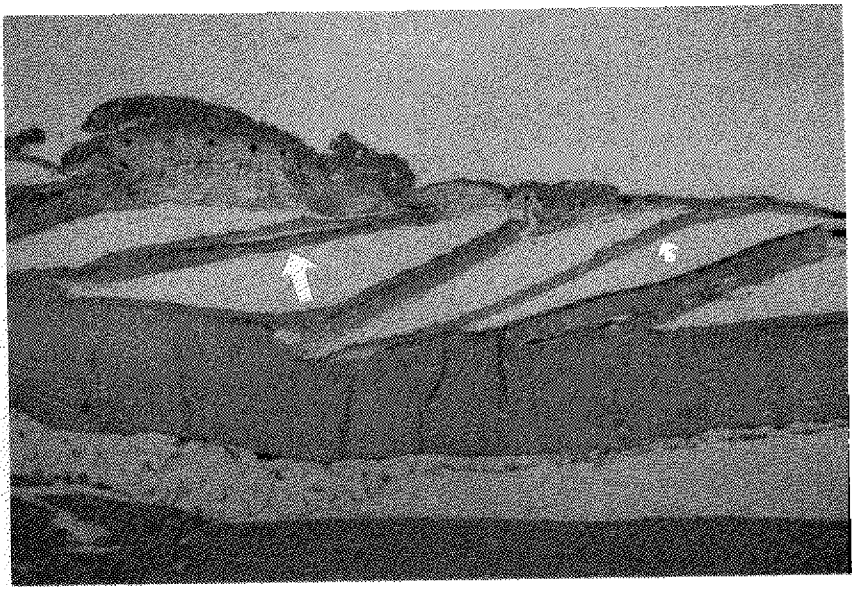

FIG. 7. Twelve hour sea lamprey wound showing scale uplifting. H & E. ×25.

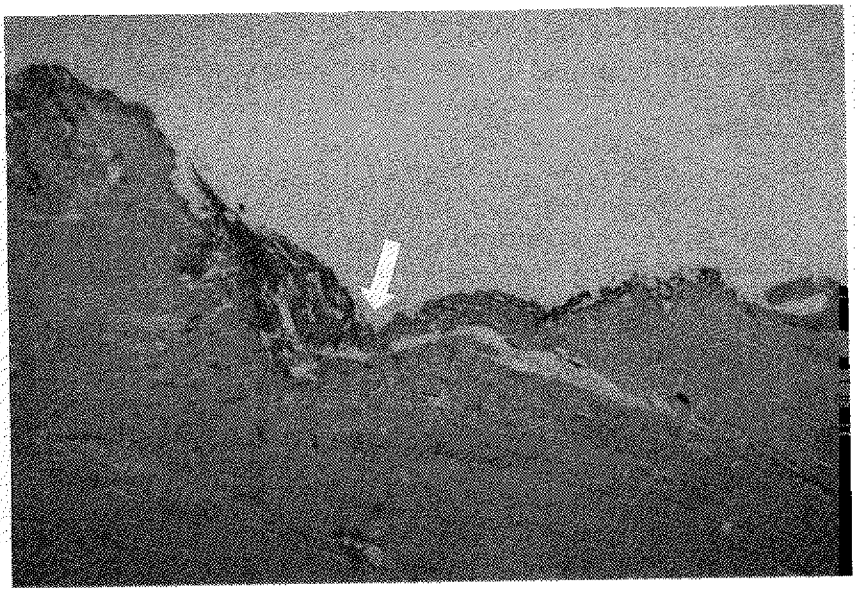

FIG. 8. Twelve hour sea lamprey wound showing area of dermal penetration. H & E. ×25.

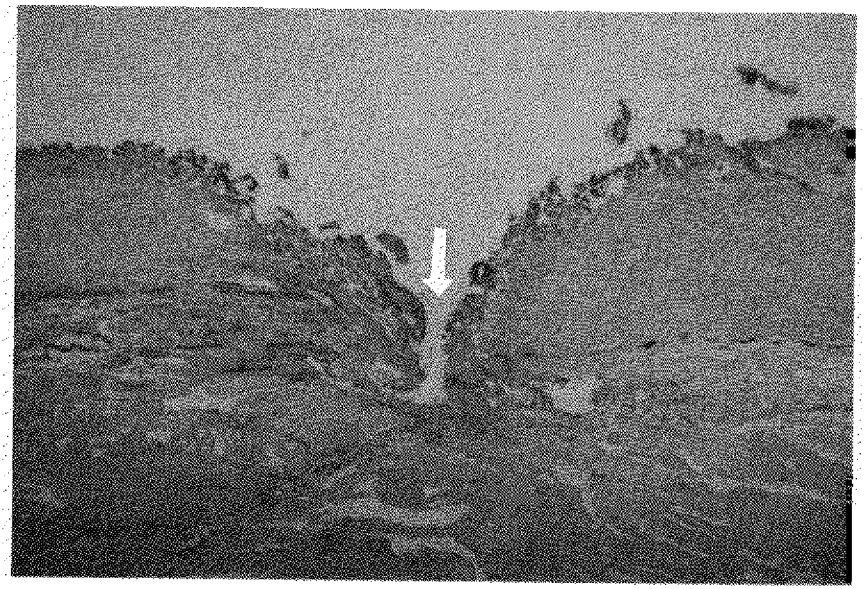

FIG. 9. Two day sea lamprey wound showing area of dermal penetration with muscle necrosis below. H & E. ×25.

from normal to hemorrhagic. Some caseation necrosis had occurred in the underlying muscle in the Type A, Stage I wound.

Two Day Wound (Type A, Stage I): Besides the changes mentioned earlier, the epidermis at the wound edge had lost its organization and the cell layers were undergoing extensive necrosis.

The dermal stratum spongiosum at this stage was absent over parts of the wound surface, and that which remained showed changes similar to those described earlier in the developing wound.

The dermal stratum compactum exhibited one major area of penetration in which caseation necrosis, accompanied by some fragmentation of collagen fibers, had occurred along this immediate edge (Fig. 9). Disruption of part of the hypodermis and some cellular infiltration were evident.

Muscle fiber degeneration occurred around the dermal break (Fig. 10). Various stages of muscle necrosis were present accompanied by edematous separation of muscle fibers, a hyaline appearance of muscle fasciculi, sarcoplasmic dissolution, and caseation. Some cellular infiltration and hemorrhage had occurred in the upper muscle layers.

Ten Day Wound (Type A, Stage I): In addition to the previously described epidermal changes, the epidermis at the wound edge exhibited a high degree of intercellular edema which had progressed to the point where the epidermal

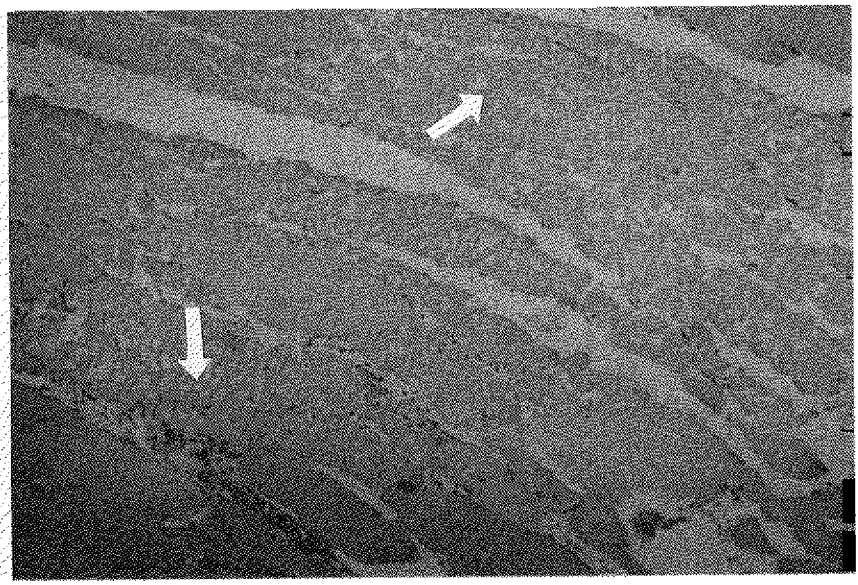

FIG. 10. Muscle necrosis in a 2-day wound by a sea lamprey. H & E. ×40.

elements appeared vacuolated. The mucous cells were generally concentrated near the surface. Scales were absent through the wound area, however, some were uplifted and broken near the wound edge.

The dermal stratum spongiosum was partially to completely absent throughout the wound area, and that which remained had changes similar to those described for earlier stages. Cellular infiltration had occurred in parts of the stratum spongiosum with polymorphonuclear leucocytes predominating.

The dermal stratum compactum was absent throughout a large portion of the wound area exposing the underlying muscle. In addition to previously mentioned lesions, many areas of the stratum compactum near the wound edge exhibited broken collagen fibers while in several areas their arrangement was disrupted. Cellular infiltration occurred near the stratum compactum. Hemorrhage and cellular infiltration, with polymorphonuclear leucocyte predominance, occurred within the hypodermis.

Degeneration of muscle fibers occurred in the area of the wound and the various stages of muscle necrosis present in the 2-day wound were also present but more widespread. Myophagia was evident in some areas of the necrotic muscle and hemorrhage, resulting from the destruction of capillaries, was present in the area of muscle damage. Cellular infiltration occurred with heavy polymorphonuclear leucocyte predominance.

The histopathological observations made of the wound development gave an excellent example of the type of damage a sea lamprey inflicts on a fish and a summary is given in Table 1.

TABLE 1. General trends in the histopathology of sea lamprey inflicted wounds on rainbow trout over a sequential time period.

Wound development stage	Epithelium	Scales	Dermal stratum spongiosum	Dermal stratum compactum	Hypodermis	Muscle
4 h	-absent	-absent	-necrosis	-normal	-normal	-normal
12 h	-absent	-absent -uplifted	-necrosis	-superficial necrosis	-normal	-normal
2 days	-absent	-absent	-absent	-necrosis along area of penetration	-disruption -cellular infiltrate	-necrosis, hemorrhage, and cellular infiltrate in upper area
10 days	-absent	-absent	-absent -cellular infiltrate	-absent -cellular infiltrate	-disruption -hemorrhage -cellular infiltrate	-necrosis -hemorrhage -cellular infiltrate -myophagia

Lennon (1954) observed that fish skin is affected even by a brief attachment of sea lamprey; incomplete attachments interrupt the mucous coat and scales and abrade the skin. He found that damage to muscle tissues often appeared greater than could have been caused by the suction action and rasping tongue. Besides mechanical injury, he demonstrated a cytolytic or histotoxic property in the secretions of the sea lampreys' buccal glands by injecting small amounts of these secretions subcutaneously in living fish. Reactions were observed in various species of trout and sucker and some died. The integument appeared to be the most resistant and the muscle and vascular tissue the least resistant. In our study the stratum compactum of the integument was also the most resistant and was only penetrated by the mechanical injury induced by the rasping tongue of the lamprey. The resistance of this part of the dermis could be attributed to its layers of collagen. Once the dermis was penetrated, muscle necrosis began to spread. Muscle necrosis occurred away from the penetration area thus indicating the histolytic actions of the buccal gland secretions. Thus many of the changes described for the developing wound were the result of some mechanical damage inflicted by the feeding lamprey and histolytic effects caused by the digestive-like secretions from the lamprey.

Some of the epidermal pathology that was evident in our study was presumably caused by the buccal secretions of the sea lamprey. Roberts and Bullock (1976) noted in marine teleost fish that the development of intercellular edema and the separation of epidermal malpighian cells by fluid from the dermis were the earliest indications of an inflammatory response in the epidermis. The formation of globules of basophilic nucleic acid throughout the affected area was caused by disordered epidermal metabolism which caused necrosis of the malpighian cells. They observed karyolytic changes of the cells and ultimately a complete dissolution of the cytoplasm and rupture of the nuclear membrane. They determined that any agent interfering with the metabolism of the epidermis resulted in this type of acute inflammation but it was most commonly caused by toxins produced by gram-negative microorganisms.

The stratum spongiosum is a complex upper layer of the dermis which includes the scales, pigment cells, mast cells, and fine fibers which bind the epidermis. Disruption of this layer has detrimental effects on the overlying epidermis (Roberts and Bullock 1976). They also described the stratum compactum as a dense layer of collagen penetrated by nerves and blood vessels which serve the stratum spongiosum. The hypodermis, which is a relatively loose tissue with a rich supply of blood vessels, is probably the region where the lamprey first begins to obtain a rich blood supply for nourishment.

In wounds that were created by sea lamprey in our study, the stratum spongiosum underwent caseation necrosis and was stained a basophilic color. Similar results were reported for other types of wounds by Roberts et al. (1973b) and Roberts and Bullock (1976) who found that integument which had been ulcerated for any length of time developed hyalinized superficial dermal collagen bundles, and had a bluish sheen with loss of fibroblast nuclei demonstrated in H

& E stained sections. This was known as the "water effect," and they suggested that it probably results from an alteration in the ionic charge on collagen which had been disrupted after exposure to water. They found that this layer of collagen is sequestered by macrophages after epithelialization before reestablishment of the stratum spongiosum.

The inflammatory response in the lamprey wound was evident after a 10-day attack, but some signs were evident as early as 2 days. Many of the cells were identified as polymorphonuclear leucocytes; others were not so easily identified but because of their abundant cytoplasm and unlobed nuclei they were assumed to be macrophages. An early infiltration of neutrophils in injured fish tissue was reported by Ellis (1977). A neutrophil response has also been described for muscle injury caused by tagging salmon parr (Roberts et al. 1973a), aeromonad infections in brown trout, *Salmo trutta* (Thorpe and Roberts 1972), and infections caused by lernaeid copepods in white bass, *Morone chrysops* (Joy and Jones 1973). Finn and Nielson (1971a, 1971b) found that neutrophilia occurred in 24 h after intraperitoneal injection of adjuvant in rainbow trout and lasted for less than a day, whereas Hines and Spira (1973) demonstrated that experimental infection of the mirror carp (*Cyprinus carpio*) with *Ichthyophthirius multifiliis* caused neutrophilia to occur within 48 h and to last about 8 days.

Roberts and Bullock (1976) reported that the polymorphonuclear leucocytes play a minor role and that the predominant cell, even in acute inflammatory responses, was the macrophage which could be of tissue or monocyte origin. They also reported that macrophages were present in inflammatory foci in early stages of their development and that they are very phagocytic toward damaged melanophores of the stratum spongiosum.

Wound Healing

Gross Pathology

Wounds at Lamprey Detachment (Type A, Stage I): The wounds were elevated with the central portion exhibiting a lesion with a mean diameter of 4.0 by 6.0 mm where the lamprey's tongue penetrated the dermis to the muscle (Fig. 11). These dermal penetrations ranged from 1.0 by 1.0 mm to 6.0 by 15.0 mm across and only two of twenty fish lacked complete dermal penetration at the time of lamprey detachment (Type B, Stage I). Some ulceration into the necrotic muscle occurred in the larger dermal openings and the muscle varied from pale to dark and was, at times, hemorrhagic. A white abraded area, which at times had some grey or brown pigmentation remaining, surrounded the wound. At times multiple perforations through the dermis to the muscle were evident. Hemorrhaging, mainly petechial, along the with some erythema was evident on many oral disk regions of the wound.

Two Days (Type A, Stage I): The elevation of wounds decreased and some sloughing of tissue in the white abraded central region was evident, especially around the dermal penetration. Most of the necrotic muscle was pale and at times

FIG. 11. Eight day sea lamprey wound on a rainbow trout.

hemorrhagic. Petechial hemorrhages and erythema were present in the peripheral portion of the wound area.

One Week (Type A, Stage I): A decline in the elevation of the wounds was evident and the area of dermal penetration expanded by 1.0 to 2.0 mm (Fig. 12). Most of the necrotic muscle was dark and somewhat hemorrhagic. The area surrounding the wound frequently became paler and at times had grey or brown pigmentation remaining. Small perforations through the dermis which were present in this area were slightly enlarged. Hemorrhaging, mainly petechial, along with erythema, was also apparent in the peripheral portion of the wounds on many fish.

Two Weeks (Type A, Stage II): Wound elevation was no longer evident on any of the fish and the area of dermal penetration had enlarged slightly. Most of the necrotic muscle visible through the dermal opening was dark and this ulcerated area in the muscle was shallower than at 1 week. The periphery of the wound area was white and had petechial hemorrhages. The brown pigmentation in wounds had been replaced by a grey color. One fish that lacked a complete dermal penetration had a dark grey to black pigmentation returning in most of the wound area (Type B, Stage II).

Three Weeks (Type A, Stage II): The area of dermal penetration closed an average of 1.0 mm from the previous week. A glassy covering was evident over

FIG. 12. Eight day sea lamprey wound after 1 week of healing.

the wound area in many of the fish and dark necrotic muscle was present beneath in the dermal opening. The periphery of the wound was grey, and at times brown pigments had moved into the whitened area from the wound perimeter. The small dermal perforations that were present in this area in some of the fish had started to close and most of the petechial hemorrhage was no longer evident. When the wound occurred on the less pigmented ventral surface of the fish, the color of the wound area appeared lighter than that of the surrounding area. The wounds that lacked complete dermal penetration were dark grey to black and some scales had reestablished (Type B, Stage III).

Four Weeks (Type A, Stage III): The wound closed another 0.5 to 3.0 mm from the previous week (Fig. 13). In many of the fish a glassy covering was present over the wound area which had a greyish sheen over the once exposed muscle. At times, the muscle was no longer visible through the dermal opening. The periphery of the wound was dark grey or brown.

Weeks Five through Six (Type A, Stage III): The wound closed another 0.5 to 2.0 mm since week 4 (Fig. 14). The once exposed muscle was entirely covered by a glassy covering. Dark grey or grey-brown pigment had filled in the wound area except for a few areas that remained white. Some fish had reestablished scales in the wound area.

Weeks Eight through Ten (Type A, Stage IV): A greyish indentation averaging 1.5 by 3.0 mm across was all that remained where the lamprey tongue

FIG. 13. Eight day sea lamprey wound after 4 weeks of healing.

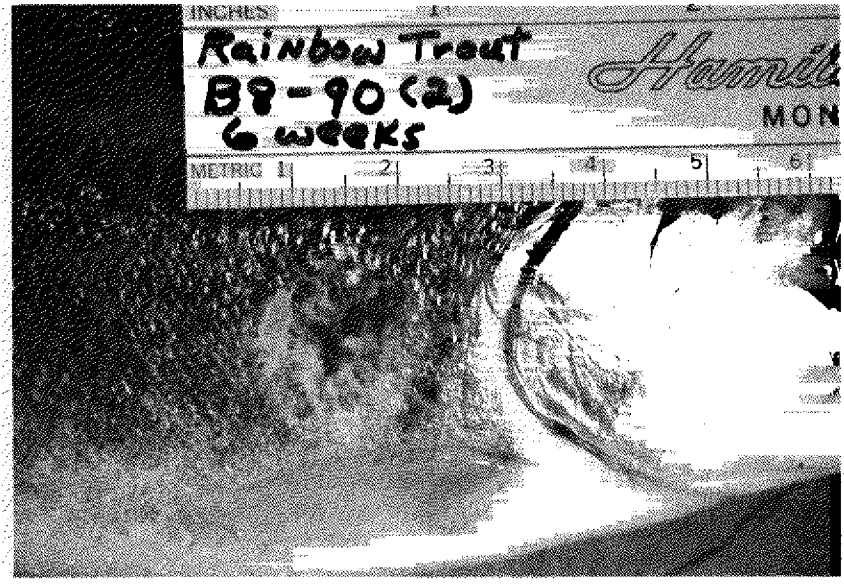

FIG. 14. Eight day sea lamprey wound after 6 weeks of healing.

FIG. 15. Eight day sea lamprey wound after 12 weeks of healing.

had penetrated through the dermis. The periphery of the wound area was more densely pigmented with a grey covering over nearly all the wound area. Much of the wound area lacked scales but new scales were forming at the periphery.

Weeks Twelve through Thirteen (Type A, Stage IV): A grey shallow indentation averaging 1.3 by 2.5 mm across remained where the lamprey tongue had penetrated the dermis (Fig. 15). The wound had regained most of its grey pigmentation but scales were lacking in much of the wound area. Wounds in the non-pigmented region of the body were lighter in color than the surrounding areas. The fish that lacked a complete dermal penetration had a slightly darker grey pigmentation in the wound area compared with the surrounding area (Type B, Stage IV).

Sea lampreys can move over the body of the host fish while attached to it. In one instance, a sea lamprey moved about 8.0 cm from the mid-region to behind the operculum within 24 h, leaving a dark grey to brown trail which was void of scales. In 2 weeks, the trail appeared to be bleached and white (Fig. 16). By week 5, the trail showed grey pigmentation and reestablishment of scales, and by week 7, it was not noticeably different from the surrounding area.

At the time of lamprey detachment, many wounds were elevated and had an average diameter of 4.0 by 6.0 mm at the point of dermal penetration. Only two fish did not show complete dermal penetration at the time of lamprey detachment. Similar incomplete wounds, as noted by Lennon (1954) and King and

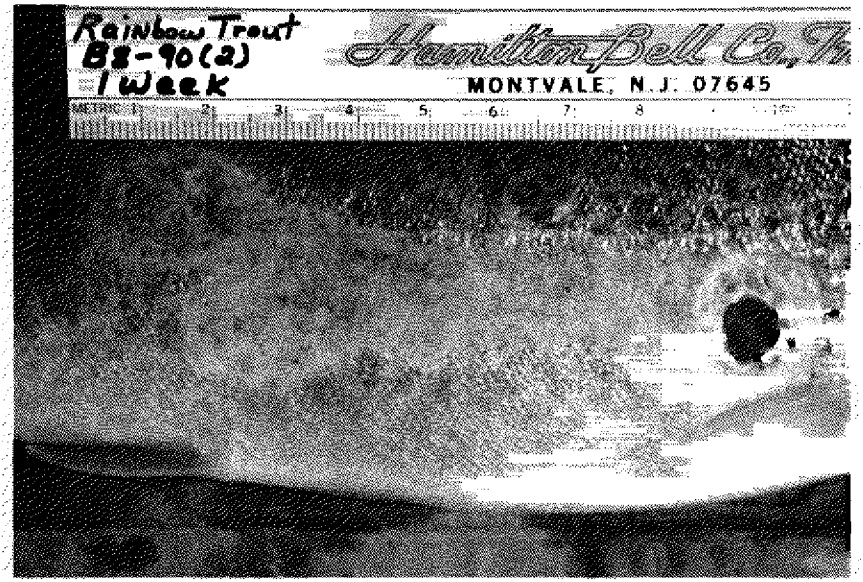

FIG. 16. Area of sea lamprey movement over the host's body 2 weeks after initial movement.

Edsall (1979), varied from temporary marks on the surface of the fish to wounds in which the scales had been torn but the skin had not been penetrated.

HISTOPATHOLOGY

The fish used in the wound healing study were all subjected to an 8-day sea lamprey attachment and most of the wounds after lamprey detachment were classified as Type A, Stage I. Only two of twenty fish lacked complete dermal penetration in the wound area at the time of lamprey detachment (Type B, Stage I).

Two Days (Type A, Stage I): Most of the epithelial elements were absent over the wound area and the elements that remained exhibited a high degree of spongiosis. Limited squames or ribbons of epitheleum occurred in the outer wound area (Fig. 17). In one wound sample epithelialization which exhibited a high degree of spongiosis was evident over two areas where dermal disruption occurred. Some of the newly formed epidermis was ragged, vacuolated in appearance, and the mucous cells occupied the upper one third (Fig. 18). Cellular infiltration was present with many polymorphonuclear leucocytes. Epithelial hyperplasia and intercellular edema accounted for the extra thickness of the epidermis over the wound area.

Scales on the 2-day healed wounds were either absent or scarce. The dermal stratum spongiosum was either partially absent or almost completely absent

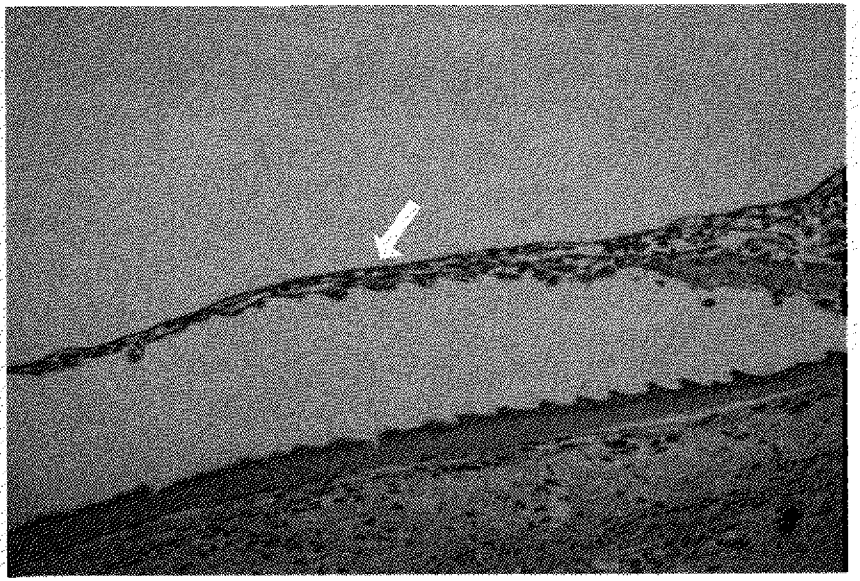

FIG. 17. Epithelialization after 2 days of wound healing. H & E. ×100.

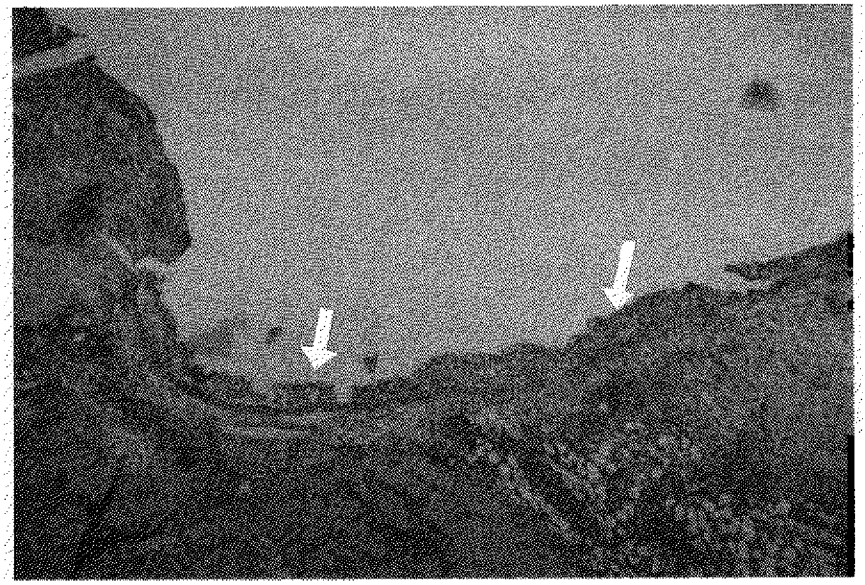

FIG. 18. Epithelialization over dermal penetration after 2 days of wound healing. Note the concentration of mucous cells near the surface of the newly formed epidermis. H & E. ×25.

throughout the wound area and much of what was present had undergone caseation necrosis and was basophilic. Heavy cellular infiltration resulted in some phagocytosis of melanin from the melanophore fragmentation. Fungal and heavy bacterial invasion of the stratum spongiosum was evident in one wound.

The dermal stratum compactum was absent in a large area where the wound entered the exposed muscle. The degree of structural organization of the stratum compactum through the wound area had deteriorated. Caseation necrosis was predominant along the edge of the dermal penetration. Cellular infiltration varied from moderate to heavy throughout the stratum compactum.

Cellular infiltration and vascular congestion ranged from moderate to heavy in the hypodermis in the wound area. One of five wounds had heavy bacterial colonization in the hypodermal region of the dermal penetration.

A large area of muscle necrosis was present throughout the wound area. Various stages of muscle necrosis were present accompanied by edematous separation of muscle fibers, a hyaline appearance of muscle fasciculi, sarcoplasmic dissolution, and caseation. Much of the muscle lining the pitted wound area was vacuolated. Hemorrhage and cellular infiltration with occasional heavy focal accumulations of cellular infiltrate occurred in the damaged muscle. Edema fluid or sarcoplasm was evident between some of the muscle bundles and myophagia was evident. Containment of necrotic muscle occurred along fascial planes which delineated necrotic muscle within the wound area from that of normal muscle on the wound periphery. One of five wounds had infiltration of rod shaped bacteria into the necrotic muscle and phagocytosis of some of the bacteria was evident (Fig. 19).

Two Weeks (Type A, Stage II): Epithelialization occurred throughout the wound area and covered the area through the dermal opening over the necrotic muscle (Fig. 20). Extensive spongiosis existed in the newly formed epidermis and mucous cells were concentrated on the surface. Scales were absent throughout the wound area but some scale regeneration was evident near the wound edge.

The dermal stratum spongiosum ranged from partially absent to almost completely absent throughout the wound area. The stratum spongiosum that was present had changes similar to those described for wound healing at 2 days and also exhibited some fibroblastic activity. The dermal stratum compactum was absent in the middle of the wound area and that which was present had changes similar to those described at 2 days wound healing. It showed some fibroblastic activity in the wound area and fragmentation of collagen fibers occurred at several points. One wound (Type B, Stage II) had no interruption of the stratum compactum and exhibited some caseation necrosis and dissolution of collagen fibers in a few isolated areas of the upper stratum compactum.

Heavy fibroblastic activity was evident in the hypodermis, especially in the area near the dermal penetration, and fibrogranulation was present throughout this area.

A large area of muscle necrosis was present throughout the wound area except in one sample that had an intact stratum compactum and contained normal

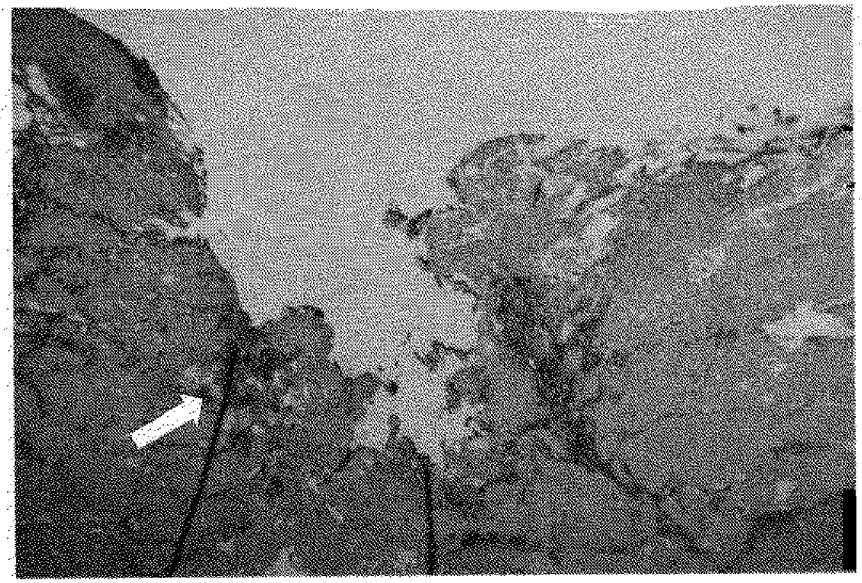

FIG. 19. Bacterial infiltration into necrotic muscle 2 days following lamprey detachment. Essentially no healing has occurred in the wound area. H & E. ×25.

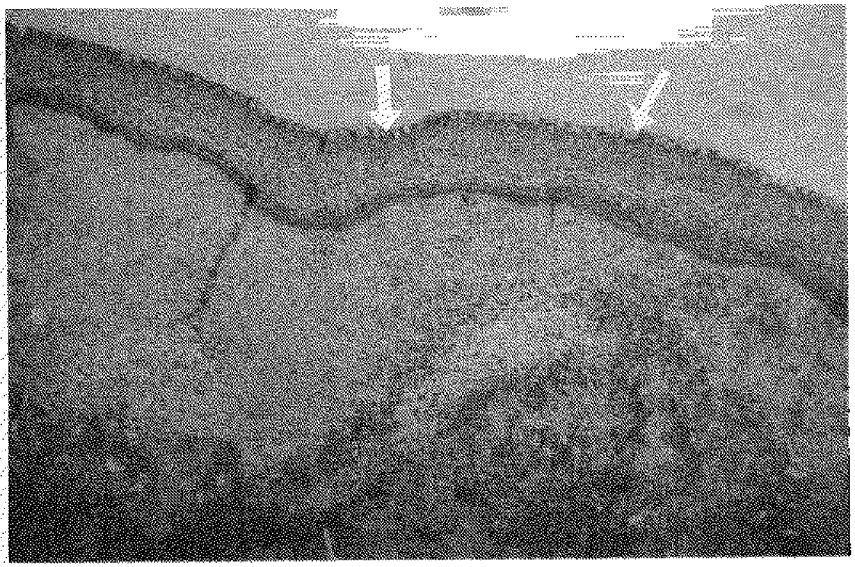

FIG. 20. Epithelialization over necrotic muscle after 2 weeks healing. Note the concentration of mucous cells near the surface of the newly formed epidermis. H & E. ×25.

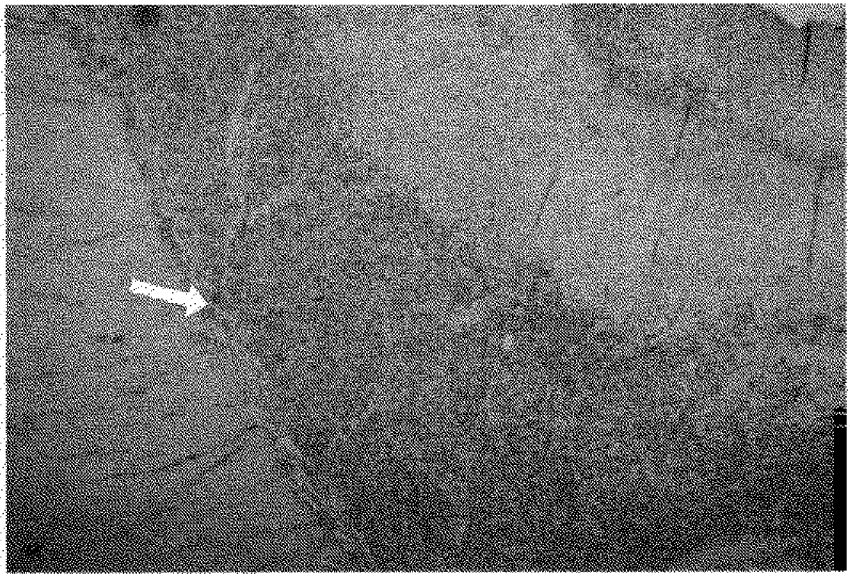

FIG. 21. Fibrogranulation and muscle necrosis contained by muscle fascial plane after 2 weeks of wound healing. H & E. ×40.

muscle (Type B, Stage II). A caseation-type necrosis was evident throughout most of the damaged muscle with resultant loss of individual fascicular structures and muscle striations. Heavy fibroblastic activity and cellular infiltration were evident. Fibrogranulation and neovascularization were present in the area of muscle necrosis, and myophagia was exhibited in some areas. Fibrogranulation and necrotic muscle were contained along fascial planes (Fig. 21). Muscle regeneration in the form of many round basophilic buds of varying sizes with large nuclei was evident in one wound (Fig. 22).

Formation of granulation tissue in the damaged muscle extended under the stratum compactum into the hypodermis near the dermal penetration and was confirmed by a trichrome stain.

One Month (Type A, Stage III): Epidermis over the wound area exhibited spongiosis and the epithelial cells were disorganized. Mucous cells were absent or few in the wound center and those present were usually in the upper half of the epidermis. Scales were absent through most of the wound area. Some scale regeneration was present near the wound edge although one sample had some regeneration through the center of the wound (Fig. 23).

The dermal stratum spongiosum ranged from present to absent closer to the center of the wound and when present was disorganized. Reorganization was evidenced by increased fibroblastic activity.

The dermal stratum compactum lacked full architectural organization and

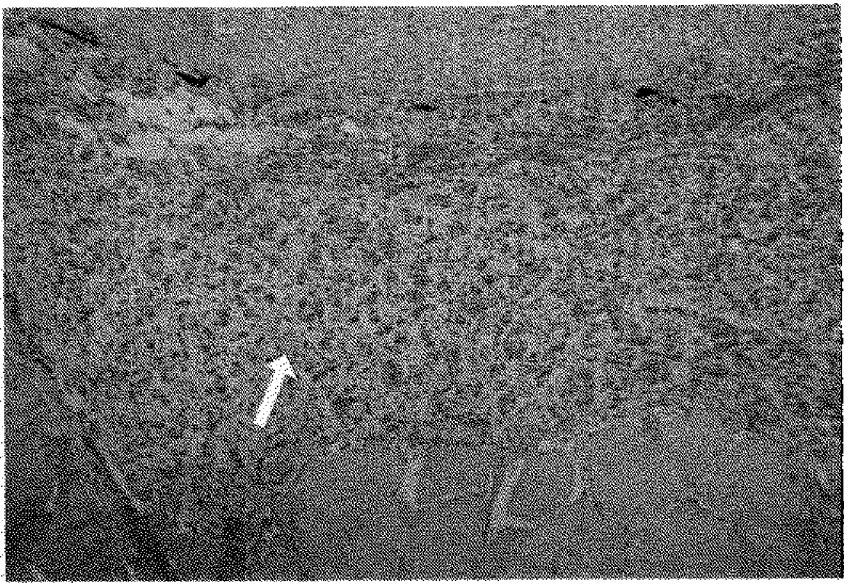

FIG. 22. Muscle regeneration present after 2 weeks of wound healing. H & E. $\times 40$.

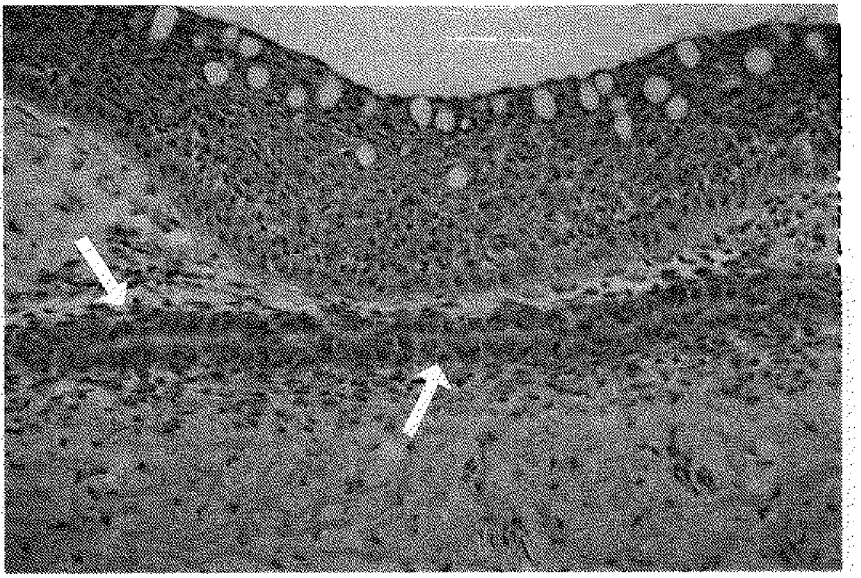

FIG. 23. Scale regeneration after 1 month of wound healing. H & E. $\times 100$.

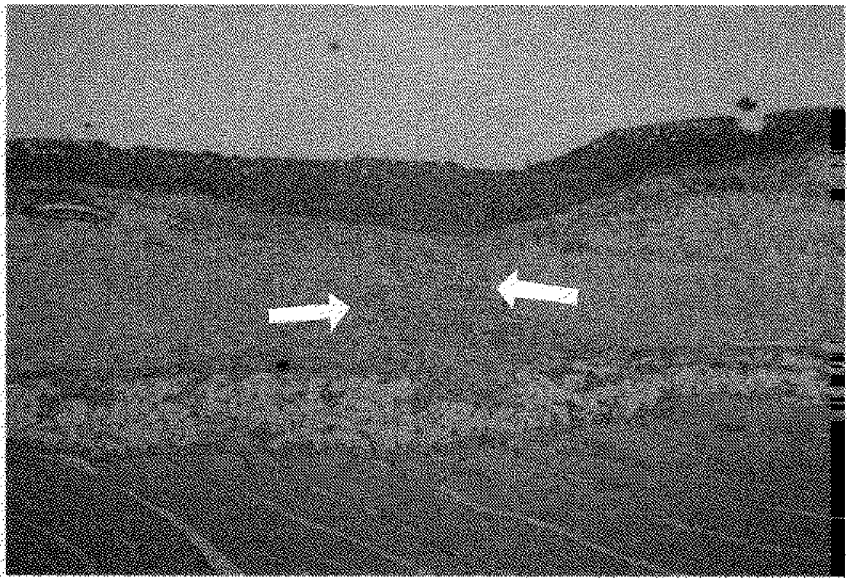

FIG. 24. Wound area after 1 month of healing showing dermis interrupted by area of fibrogranulation where lamprey tongue penetrated earlier. H & E. × 25.

had an area interrupted by fibrogranulation (Figure 24). Heavy fibroblastic activity was most predominant near the center of the wound area. Some fibrogranulation was evident in the hypodermis near the wound center.

The muscle varied in appearance from normal to having fibrogranulation which encompassed the area of muscle damage. Neovascularization was evident throughout the granulation tissue and the fibrogranulation was contained by the fascial planes. Regenerating muscle fibers having a blueish hue and large muscle fiber nuclei were evident. Many small muscle bundles with irregularly arranged fibers were observed (Fig. 25).

Three Months (Type A, Stage IV): Normal epidermis with its complement of mucous cells was present over the wound area (Fig. 26). Although some scale regeneration was evident, scales were absent through most of the wound area except in one sample where they were present (Type B, Stage IV).

Changes occurring in the dermal stratum spongiosum showed wide variation. One sample, a Type B, Stage IV wound, was normal in appearance but in others the stratum spongiosum was absent or somewhat disorganized. Continued reorganization was evidenced by the fibroblastic activity in the stratum spongiosum.

The stratum compactum of the dermis was normal in the Type B, Stage IV wound but another sample still showed an area interrupted by fibrogranulation. The collagen fibers were disorganized in a few samples. The presence of

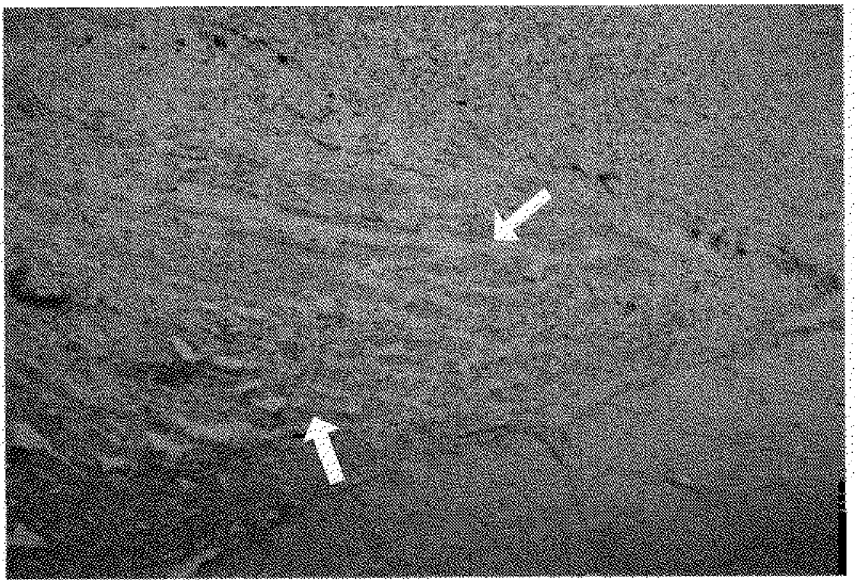

FIG. 25. Muscle regeneration after 1 month of wound healing. H & E. ×40.

fibroblastic activity was evidence of continued tissue reorganization (Fig. 26). The hypodermis was normal in all samples except one which showed some fibrogranulation where the stratum compactum was interrupted by granulation tissue.

The muscle was normal in all samples except one. In that sample limited fibrogranulation and neovascularization occurred in the muscle immediately below the area of the stratum compactum that was interrupted by granulation tissue. Muscle fasciculi were disorganized in this area and most exhibited separation of muscle fibers.

A summary of the histopathology of wound healing is given in Table 2.

DISCUSSION OF WOUND HEALING

Epithelialization of lamprey-inflicted wounds was similar to that reported for wound healing in fish by Roberts et al. (1971), Mawdesley-Thomas and Bucke (1973), Mittal and Munshi (1974), Anderson and Roberts (1975), Laird et al. (1975), Roberts and Hill (1976), Phromsuthirak (1977), and Bullock et al. (1978). High numbers of neutrophils were noted in the newly formed epithelial tissue of several fish with lamprey inflicted wounds. Phromsuthirak (1977), who studied the healing process of small surgical incisions in the skin of *Gasterosteus aculeatus*, noted both neutrophils and macrophages in newly formed epidermis which were phagocytic.

The pathologies described in the dermal area of healing lamprey-inflicted

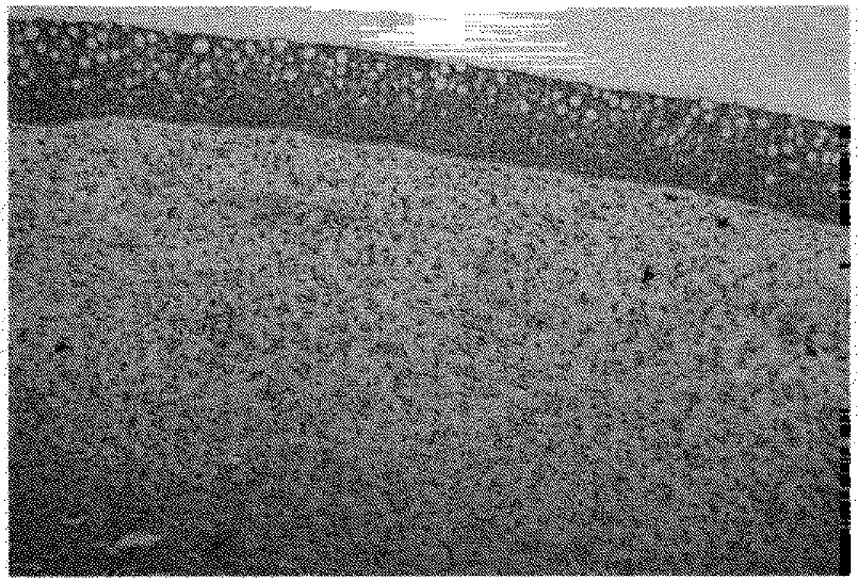

FIG. 26. Wound area after 3 months of healing. Epidermis was normal and dermal area showed fibroblastic activity. H & E. ×40.

wounds were very similar to those found by other researchers in the healing of wounds in fish. Mawdesley-Thomas and Bucke (1973) observed that goldfish which had been injured with a hypodermic needle demonstrated increased fibroblastic activity with a proliferation of collagen fibers within the dermis. They also noted that capillary congestion and hemorrhage resulted in edema between the dermis and epidermis with an increase in chronic cellular infiltration. Roberts et al. (1971) noted that dermal melanocytes usually followed the epithelial cells into the center of healing lesions in fish that had suffered from ulcerative dermal necrosis (UDN). Other studies by Roberts and Hill (1976) on brown trout recovering from UDN showed that the stratum spongiosum melanophores were present around the edge of the lesion but no melanin layer in the dermis appeared at the center until resolution was complete.

Even though sea lamprey can inflict large wounds, healing appears good if the fish is not severely anemic and does not succumb to secondary infection. The wound is closed by epithelialization by 2 weeks after lamprey detachment although the newly formed epidermis lacks the degree of organization seen in the normal epidermis. Some scale regeneration had commenced at 2 weeks after lamprey detachment and was undoubtedly dependent on the condition of the stratum spongiosum, the site from which the scales originate. The stratum spongiosum at this time had shown early signs of fibroblastic activity, an indication of reorganization. Roberts and Bullock (1976) observed that damaged scales are usually sloughed through the epidermis and a new scale grows in if

TABLE 2. General trends in the histopathology of healing wounds inflicted by sea lamprey on rainbow trout.

Wound healing stage	Epithelium	Scales	Dermal stratum spongiosum	Dermal stratum compactum	Hypodermis	Muscle
2 days	-absent -limited epithelialization	-absent	-absent -cellular infiltration	-absent -cellular infiltration	-cellular infiltration -vascular congestion	-necrosis -hemorrhage -cellular infiltrate -myophagia -containment of necrosis by fascial planes
2 weeks	-epithelialization (spongiosis)	-absent -limited regeneration	-absent -cellular infiltration -fibroblastic activity	-absent -cellular infiltration -fibroblasic activity	-cellular infiltration -fibroblastic activity	-necrosis -cellular infiltrate -myophagia -fibroblastic activity -neovascularization -containment of fibrogranulation by fascial planes -muscle regeneration
1 month	-present (spongiosis)	-absent -some regeneration	-fibroblastic activity -reorganization	-fibrogranulation	-fibrogranulation	-fibrogranulation -neovascularization -muscle regeneration
3 months	-present (normal)	-absent -some regeneration	-fibroblastic activity -reorganization	-fibrogranulation	-normal	-normal

the germinal tissue is not damaged by the trauma. Laird et al. (1975), recording the effects of freeze branding on salmon, observed that by the third week scale regeneration was prominent except directly below the site of branding.

Fibrous replacement in a lamprey-inflicted wound was heavy at 2 weeks and at 1 month following lamprey detachment, but some muscle regeneration was also evident. Finn and Nielson (1971a), Mawdesley-Thomas and Bucke (1973), and Roberts et al. (1973a) found that in damaged muscle, granulation tissue replacement was far more prominent than myofibrillar regeneration. By 3 months following lamprey detachment, the wound area was occupied by muscle with negligible fibrogranulation. Muscle regeneration in wounds of fish was confirmed by Ramachandran and Thangavelu (1969), Roberts et al. (1973b), Mittal and Munshi (1974), and Anderson and Roberts (1975). In a sea lamprey inflicted wound the perimysial fascial planes limited the spread of muscle necrosis which was also observed by many researchers studying wound healing in fish (Finn and Nielson 1971a; Roberts et al. 1973a; Anderson and Roberts 1975; Laird et al. 1975). The fibrogranulation in a lamprey-induced wound appeared to originate from the perimysial fascial planes and hypodermis within 2 weeks following detachment. Fibrogranulation originating from the perimysial plane and hypodermis in healing fish wounds was also observed by Finn and Nielson (1971a), Roberts et al. (1973a), and Laird et al. (1975).

During sea lamprey attack on a fish the inflammatory response was not as pronounced as that following lamprey detachment. This may be due to the lamprey buccal gland secretions having a suppressing effect upon cellular infiltration. The predominant inflammatory cell in a lamprey-inflicted wound was the polymorphonuclear leucocyte. Roberts et al. (1973a) who studied the histopathology of salmon tagging, found a cellular response comprised chiefly of macrophages with a few polymorphonuclear leucocytes. Anderson and Roberts (1975) compared the healing of minor surgical wounds in Atlantic salmon (*Salmo salar*) and the White Mountain cloud minnow (*Tanichthyes albonubes*) and found both polymorphonuclear leucocytes and macrophages in the wound area. Finn and Nielson (1971a) studied the effects of various injuries in rainbow trout and found that the inflammatory response was closely comparable with that of mammals, although less intense and slower to appear and resolve.

A shallow indentation remained even after 3 months of wound healing on the surface of the fish where the sea lamprey tongue had penetrated through the dermis. During wound healing little exudate was present through the area where the lamprey's tongue had penetrated the dermis and epithelialization followed the contour of the wound. Ramachandran and Thangavelu (1969) found that in *Ophiocephalus* sp. the presence of a blood clot is not an important factor in the healing of wounds. It was shown that fish exudate contains little blood and does not fill the entire wound cavity, so that in any extensive wound the scar and epithelialization usually fail to restore the original surface level.

Temperature has a direct effect on wound healing of lamprey scars on fish. Lennon (1954) observed that wound healing of lamprey scars begins sooner in the summer than in the winter and attributed this to the higher metabolic rate of fishes in warm water. Finn and Nielson (1971b) and Roberts et al. (1973a) found

the rate of development of various cellular and tissue components to be much slower at lower temperatures. They determined the lower temperatures delayed the wound healing and inflammatory response and thus slowed down events in the process leading to healing.

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